











Morphology and multi-gene phylogeny reveal three new species of *Clonostachys* and two combinations of *Sesquicillium* (Bionectriaceae, Hypocreales) from Xizang, China

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Abstract

Clonostachys and *Sesquicillium* are genera in Bionectriaceae, and known in sexual perithecial ascomata and hyphomycetous asexual morphs. In their asexual morph, both genera share similar morphology in conidiophores and conidiogenous cell characteristics but differ in the development of conidiophores. The members of *Clonostachys* are distributed worldwide with the majority occurring in the tropics and the species are commonly reported as soil-borne fungi but also reported as endophytes, epiphytes, and saprotrophs. During a microfungi survey in Xizang, China, six collections of fresh and healthy *Ageratina adenophora* and *Houttuynia cordata* leaves were obtained. The taxonomy of these collections was investigated through a combination of morphological analysis and multigene phylogenetic analysis using Maximum likelihood and Bayesian inference. The newly generated sequences were clustered within *Clonostachys* and *Sesquicillium*, showing hyphomycetes asexual morph. The results revealed three new *Clonostachys* species viz, *Clonostachys linzhiensis*, *C. motuoensis*, and *C. yadongensis*. This research sheds light on the overlooked fungal diversity in Xizang, China, expanding the known fungal biodiversity in the region. Additionally, two new combinations, *Sesquicillium aquaticum* and *S. shanghaiense* for *C. aquatica* and *C. shanghaiensis*, and one synonymy, *C. viticola* for *C. swietenia* are established, respectively.

Key words: Asexual morph, endophytes, Hyphomycetes, new taxa, taxonomy

Introduction

Clonostachys (Bionectriaceae, Hypocreales) was established by Corda (1839). The genus was typified by *C. araucaria* (Corda 1839), which was later synonymized under *C. rosea* (Rossman et al. 2013). The genus was considered as the asexual morph of *Bionectria* and both genera were also considered as conspecific in



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several studies (Luo and Zhuang 2007, 2010; Dong et al. 2023). *Bionectria* was described by Spegazzini (1918). Based on the One Fungus = One Name (1F = 1N) concept, mycologists propose the protection of the older asexual morph-typified name *Clonostachys* for this genus (Rossman et al. 2013; Dong et al. 2023). Members of *Clonostachys* occur as endophytes, entomopathogens, epiphytes, plant pathogens, soil-borne fungi, and saprotrophs, typically found on herbicolous, corticolous, lichenicolous, fungicolous, coprophilous habitats as well as on nematodes and insects (Mazen et al. 2022; Dong et al. 2023; Wang et al. 2023; Zhao et al. 2023). They are distributed globally and commonly occur in tropical regions (Schroers 2001). The sexual morph is characterized by ascomata that do not change colour in 3% Potassium Hydroxide (KOH) or 100% Lactic Acid (LA) (Luo and Zhuang 2007, 2010), perithecial or cleistothecial ascomata that are superficial on the substrate or embedded in the stroma. Ascomata are solitary or densely aggregated, subglobose to pyriform; clavate or cylindrical, sessile or short pedicellate asci, smooth or striated, aseptate to multi-septate, globose, fusiform, ellipsoid or broadly ellipsoid ascospores (Hyde et al. 2020a). The asexual members are characterized by penicillate, sporodochial and dimorphic conidiophores (primary and secondary conidiophores) with phialidic conidiogenous cells, hyaline, smooth, broadly ellipsoidal conidia with ends that are broadly rounded (Bao et al. 2023; Chen et al. 2023; Dong et al. 2023; He et al. 2023; Liu et al. 2023; Perera et al. 2023). Primary conidiophores are mononematous, either verticillium-like or narrowly penicillate, whereas the secondary conidiophores produce imbricate conidial chains that can collapse to slimy masses, particularly on sporodochia (Zhao et al. 2023).

Morphology-based identification of *Clonostachys* is challenging (Schroers et al. 1999; Abreu et al. 2014) and many species were previously placed in various genera such as *Acrostalagmus*, *Clonostachyopsis*, *Dendrodochium*, *Gliocladium*, *Gliocladochium*, *Myrothecium*, *Sesquicillium*, *Spicaria*, *Verticilliodochium*, or *Verticillium* (Schroers 2001). Rossman et al. (2001) first conducted the initial molecular investigation of *Clonostachys/Bionectria*, employing large subunit rDNA sequences, and proposed the monophyletic status. Subsequently, DNA sequences from multigenes including ITS, 28S, *rpb1*, *rpb2*, and *tef1* have been extensively employed to address the taxonomy of *Clonostachys* (Bao et al. 2023; Chen et al. 2023; Perera et al. 2023; Zhao et al. 2023). Wijayawardene et al. (2022) accepted 78 species under *Clonostachys*, while this was 50 species in Hyde et al. (2024). Zhao et al. (2023) investigated the species diversity within a collection of 420 strains of *Clonostachys* from the culture collection and personal collections at the Westerdijk Fungal Biodiversity Institute in Utrecht, the Netherlands, and identified 19 species based on phylogenetic and morphological analyses. In China, 19 *Clonostachys* species have been reported from different hosts and substrates (Bao et al. 2023; Dong et al. 2023; Perera et al. 2023; Piombo et al. 2023; Wang et al. 2023).

During the microfungi survey in China (He et al. 2024a, b, c; Thiyagaraja et al. 2024), we investigated several isolates from the leaves of *Ageratina adenophora* and *Houttuynia cordata* from Xizang, China. Multigene phylogenetic analyses combining 28S, *tef1*, *rpb2*, ITS, and *tub2* sequences, along with morphological analyses, support the establishment of three new species: *Clonostachys linzhiensis*, *C. motuoensis* and *C. yadongensis*. The introduction of these new species follows the protocols outlined in Chethana et al. (2021) and Maharachchikumbura et al. (2021). The new species are established based on detailed morphological characterization, and illustrations, along with multigene analyses of maximum likelihood (ML)

and Bayesian inference (BI). In addition, through phylogenetic analysis of *Clonostachys*, we suggest that *C. aquatica*, *C. shanghaiensis*, and *C. swieteniae* be synonymous with *Sesquicillium aquaticum*, *S. shanghaiense*, and *C. viticola*, respectively.

Materials and methods

Sample collection, isolation, and morphological characterization

Fresh and healthy leaves of *Ageratina adenophora* and *Houttuynia cordata* were collected from Medog County, Linzhi City, Xizang Autonomous Region, China from October 2021 to July 2023, and information on collection was recorded according to the Rathnayaka et al. (2024). The healthy part of the leaves was initially cleaned and cut into small pieces (5 × 5 mm). The leaf fragments were briefly soaked in a 75% ethanol solution for 30 s, followed by a 2.5% sodium hypochlorite solution for the same duration (Bhunjun et al. 2021). Afterward, they were washed thrice with sterile distilled water for 30 s. Once sterilized, the tissue fragments were allowed to air-dry on sterile filter paper and then transferred to potato dextrose agar (PDA) (Senanayake et al. 2020). The PDA plates were cultured at 25 °C for 2–5 days. Single hyphae were carefully selected from the periphery of the growing colonies and inoculated onto new PDA plates. Following 1–2 weeks of purification, a pure culture was obtained. Sporulation was induced on water agar (WA) medium. The mycelia were mounted on a slide in water using a sterile needle. A NIKON ECLIPSE Ni-U compound microscope was used to examine conidiophores and conidia of a small mass of mycelia. Micro-morphological images were captured with a DS-Ri2 camera attached to the compound microscope. The photoplates used for the figure were processed with Adobe Photoshop. The pure cultures were deposited in the Kunming Institute of Botany, the Chinese Academy of Sciences (KUNCC), Kunming, China. Specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (KUN-HKAS), Kunming, China. Facesoffungi and Index Fungorum numbers were registered following the protocols outlined in Jayasiri et al. (2015) and Index Fungorum, respectively.

DNA extraction, PCR amplification and sequencing

The mycelia growing on a PDA plate were used to extract DNA using the Tri-leaf™ Plant Genomic DNA Kit (Tsingke Biological Technology Co., Ltd in Beijing, China), following the manufacturer's instructions. The primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), T1/T22 (Research & Service 1997), EF1-983F/EF1-2218R (Carbone and Kohn 1999), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used for amplification of the internal transcribed spacer region ITS1-5.8S-ITS2 (ITS), large subunit rDNA (28S), beta-tubulin (*tub2*), translation elongation factor 1-α (*tef1*) gene and RNA polymerase II second-largest subunit (*rpb2*), respectively. The PCR was performed in a 25 µL reaction volume, comprising 21 µL PCR Mix (2 × Rapid Taq Master Mix, Vazyme Biotech Co., Ltd., Nanjing, China), 1 µL of each primer, 2 µL of DNA template. For PCR amplification conditions see Table 1. The PCR products were visualized using agarose gel electrophoresis, and those with the targeted bands were sent to Sangon Biotech Co. Ltd., Kunming, China, for sequencing. The newly generated sequences were submitted to GenBank to obtain accession numbers.

Table 1. Loci, primers, and PCR amplification conditions used in this study.

Locus	Primers	PCR amplification conditions	Reference
ITS	ITS5/ITS4	95 °C: 5 min, (95 °C: 15s, 55 °C: 15s, 72 °C: 15s) × 40 cycles	White et al. (1990); Vilgalys and Hester (1990)
28S	LR0R/LR5		
<i>tef1</i>	EF1-983F/EF1-2218R	95 °C: 5 min, (95 °C: 45s, 52 °C: 45s, 72 °C: 70s) × 35 cycles	Carbone and Kohn (1999)
<i>tub2</i>	T1/T22	95 °C: 5 min, (95 °C: 45s, 50 °C: 45s, 72 °C: 90s) × 35 cycles	Research and Service (1997)
<i>rpb2</i>	fRPB2-5F/fRPB2-7cR	95 °C: 5 min, (95 °C: 45s, 55 °C: 120s, 72 °C: 50s) × 35 cycles	Liu et al. (1999)

Sequence alignment and phylogenetic analyses

The sequences were assembled using Sequencing Project Management (SeqMan) software (Clewley 1995). The assembled sequences were compared with the data in GenBank to determine their close relatives. The results indicate that our specimens were closely related to species of *Clonostachys*. Reference sequences for *Clonostachys* were obtained following recent studies (Bao et al. 2023; Liu et al. 2023; Perera et al. 2023; Piombo et al. 2023; Wang et al. 2023; Zhang et al. 2023; Zhao et al. 2023) (Table 2). Each gene matrix was separately aligned using MAFFT v. 6.8 (Kato et al. 2018). The aligned datasets were manually edited using BioEdit v. 7.0.9 (Hall 1999) and then combined using SequenceMatrix v1.7.8 (Vaidya et al. 2011). The combined alignment was utilized for ML and BI analyses.

A rapid phylogenetic analysis was performed utilizing OFPT (Zeng et al. 2023) according to its standard protocol. The final phylogenetic analyses were carried out on the CIPRES Science Gateway platform (<https://www.phylo.org>), employing RAxML-HPC v.8 on XSEDE (8.2.12) for maximum likelihood (ML) estimation and MrBayes on XSEDE (3.2.7a) for Bayesian inference (BI). Phylogenetic results were represented by ML bootstrap values (MLB) equal to or greater than 70% and a posterior probability in Bayesian statistics (BYPP) equal to or exceeding 0.90. These values were displayed above each node in all resulting trees. For visualization purposes, the resulting phylograms were displayed using the FigTree v1.4.0 program. The final reorganization was accomplished using Adobe Illustrator 2020.

Results

Phylogenetic analyses

The combined 28S, *tef1*, *rpb2*, ITS, and *tub2* dataset comprised 104 taxa. *Fusarium acutatum* (CBS 402.97) and *Nectria cinnabarina* (CBS 279.48) were selected as out-group taxa (Prasher and Chauhan 2017; Lechat et al. 2020). The dataset consisted of 3146 total characters, including gaps (28S: 1–784 bp; *tef1*: 785–1596; *rpb2*: 1597–2349; ITS: 2350–2826; *tub2*: 2827–3828). The matrix had 1079 distinct alignment patterns, with 41.89% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.229764, C = 0.268281, G = 0.268313, T = 0.233642; substitution rates: AC = 1.37920, AG = 4.09491, AT = 1.37920, CG = 0.794178, CT = 8.784537, GT = 1.00000; gamma distribution shape parameter α = 0.494958. The best-scoring RAxML tree with a final likelihood value of -23046.167770 is presented in (Fig. 1). Our specimens *Clonostachys linzhiensis* (HKAS 133179 & HKAS 133180) and *C. motuoensis* (HKAS 133181 & HKAS 133182) formed distinct monophyletic clades with *C. aranearum* with support value of (75% ML) and (85% ML), indicating they are closely related. The two specimens HKAS 133183 and HKAS 133184 formed a sister clade to *C. krabiensis* with high support (100 ML/0.91 PP).

Table 2. Names, voucher numbers, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses in this study.

Taxa	Voucher no.	GenBank accession numbers					Reference
		ITS	28S	tub2	tef1	rpb2	
<i>Clonostachys agrawalii</i>	CBS 533.81	AF358241	N/A	AF358187	N/A	N/A	Schroers (2001)
<i>C. ambigua</i>	PAD S00003	MT554898	N/A	N/A	N/A	N/A	Forin et al. (2020)
<i>C. apocyni</i>	CBS 130.87	AF210688	N/A	AF358168	N/A	N/A	Schroers (2001)
<i>C. aranearum</i>	QLS 0625	NR_164542	N/A	KU212400	N/A	N/A	Chen et al. (2016)
<i>C. artemisiae</i>	MHZU 23-0116	OR365451	N/A	OR700206	N/A	N/A	Dong et al. (2023)
<i>C. aurantiaca</i>	CBS:124757	OQ910531	OQ910890	N/A	OQ944545	OQ927609	Zhao et al. (2023)
<i>C. aureofilvella</i>	CBS 195.93	AF358226	N/A	AF358181	N/A	N/A	Schroers (2001)
<i>C. australiana</i>	CBS:102421	OQ910540	OQ910899	OQ982584	OQ944554	OQ927618	Zhao et al. (2023)
<i>C. bambusae</i>	CBS:139411	OQ910542	OQ910901	OQ982586	OQ944556	OQ927620	Zhao et al. (2023)
<i>C. buxicola</i>	CBS:102419	OQ910544	OQ910903	OQ982588	OQ944558	OQ927622	Zhao et al. (2023)
<i>C. byssicola</i>	CBS 364.78	MH861151	MH872912	AF358153	N/A	N/A	Vu et al. (2019)
<i>C. capitata</i>	CBS 218.93	AF358240	MH874054	AF358188	N/A	N/A	Schroers (2001)
<i>C. catenulata</i>	CBS 154.27	NR_165993	NG_063969	N/A	OQ944810	OQ927866	Zhao et al. (2023)
<i>C. chlorina</i>	CBS 287.90	NR_137651	MH873895	OQ982590	OQ944560	OQ927624	Schroers (2001)
<i>C. chloroleuca</i>	CBS:141588	OQ910549	OQ910908	N/A	OQ944563	OQ927627	Zhao et al. (2023)
<i>C. chongqingensis</i>	HMAS 290894	OP205475	N/A	OP205324	N/A	N/A	Zeng and Zhuang (2022)
<i>C. compactiuscula</i>	CBS:123759	OQ910563	OQ910922	OQ982603	OQ944576	OQ927640	Zhao et al. (2023)
<i>C. compactiuscula</i>	CBS 913.97	AF358245	N/A	AF358194	N/A	N/A	Schroers (2001)
<i>C. cylindrica</i>	CBS:101113	OQ910569	OQ910928	N/A	OQ944582	OQ927646	Zhao et al. (2023)
<i>C. divergens</i>	CBS 967.73	NR_137532	OQ910934	AF358191	OQ944587	N/A	Schroers (2001)
<i>C. ellipsoidea</i>	CBS 175.76	OQ910580	OQ910939	OQ982617	OQ944592	OQ927655	Zhao et al. (2023)
<i>C. epichloe</i>	CBS 101037	AF210675	OQ910940	AF358209	OQ944593	OQ927656	Schroers (2001)
<i>C. eriocamporesiana</i>	MFLU 18-2713	MN699132	N/A	MN699965	MN699964	N/A	Hyde et al. (2020b)
<i>C. eriocamporesii</i>	MFLU 19-0486	MN699133	NG_068919	OQ982619	N/A	N/A	Hyde et al. (2020b)
<i>C. farinosa</i>	CBS 914.97	AF358252	N/A	AF358151	N/A	N/A	Schroers (2001)
<i>C. flava</i>	CBS 915.97	OQ910619	OQ910978	OQ982654	OQ944631	OQ927690	Zhao et al. (2023)
<i>C. fujianensis</i>	CBS:127474	OQ910620	OQ910979	OQ982655	OQ944632	OQ927691	Zhao et al. (2023)
<i>C. fusca</i>	CBS 207.93	OQ910622	OQ910981	OQ982657	OQ944634	OQ927693	Zhao et al. (2023)
<i>C. garysamuelsii</i>	CBS:123964	OQ910624	OQ910983	OQ982658	OQ944636	OQ927695	Zhao et al. (2023)
<i>C. grammicospora</i>	CBS 209.93	NR_137650	NG_064165	AF358206	OQ944637	N/A	Forin et al. (2020)
<i>C. grammicosporopsis</i>	CBS 102834	AF358256	OQ910985	OQ982660	OQ944638	OQ927697	Vu et al. (2019)
<i>C. granuligera</i>	PAD S00011	MT554904	N/A	N/A	N/A	N/A	Forin et al. (2020)
<i>C. hongkongensis</i>	CBS:115291	OQ910630	OQ910989	OQ982663	OQ944642	OQ927700	Zhao et al. (2023)
<i>C. impariphialis</i>	HMAS 275560	KX096609	KX096606	N/A	N/A	N/A	Zeng and Zhuang (2022)
<i>C. indica</i>	RKV2015	KT291441	N/A	N/A	N/A	N/A	Prasher and Chauhan (2017)
<i>C. intermedia</i>	CBS 508.82	NR_137652	OQ910991	AF358205	OQ944644	N/A	Schroers (2001)
<i>C. kowhai</i>	CBS 461.95	NR_154748	OQ910992	AF358170	OQ944645	OQ927702	Schroers (2001)
<i>C. krabiensis</i>	MFLU 16-0254	NR168189	MH376707	N/A	N/A	N/A	Tibpromma et al. (2018)
<i>C. krabiensis</i>	CBS 192.96	OQ910634	OQ910993	OQ982666	OQ944646	OQ927703	Zhao et al. (2023)
<i>C. kunmingensis</i>	YFCC: 898	MW199069	MW199058	MW201676	MW295969	N/A	Wang et al. (2023)
<i>C. leptoderma</i>	HMAS 255834	OP205474	N/A	OP205323	N/A	N/A	Zeng and Zhuang (2022)
<i>C. leucaenae</i>	MFLU 20-0008	ON230050	ON230058	N/A	N/A	N/A	Perera et al. (2023)
<i>C. levigata</i>	CBS 948.97	AF210680	N/A	AF358196	N/A	N/A	Schroers (2001)
<i>C. linzhiensis</i>	HKAS 133179	PQ522504	PQ634391	PQ650459	PQ650477	N/A	present study
<i>C. linzhiensis</i>	HKAS 133180	PQ522505	PQ634392	PQ650460	PQ650478	N/A	present study
<i>C. longiphialidica</i>	CBS 112.87	OQ910643	OQ911002	N/A	OQ944655	OQ927712	Zhao et al. (2023)
<i>C. lucifer</i>	CBS 100008	AF210683	OQ911003	AF358208	OQ944656	OQ927713	Schroers (2001)
<i>C. miodochialis</i>	CBS 997.69	NR_137649	NG_064076	AF358210	OQ944658	OQ927715	Schroers (2001)
<i>C. moreaui</i>	CBS:127881	OQ910647	OQ911006	OQ982678	OQ944659	OQ927716	Zhao et al. (2023)
<i>C. motuoensis</i>	HKAS 133181	PQ522506	PQ634393	PQ650461	PQ650479	N/A	present study
<i>C. motuoensis</i>	HKAS 133182	PQ522507	PQ634394	PQ650462	PQ650480	N/A	present study
<i>C. oblongispora</i>	CBS 100285	AF358248	OQ911007	AF358169	OQ944660	OQ927717	Schroers (2001)
<i>C. obovatispora</i>	CBS:118752	OQ910649	OQ911008	OQ982680	OQ944661	OQ927718	Zhao et al. (2023)

Taxa	Voucher no.	GenBank accession numbers					Reference
		ITS	28S	tub2	tef1	rpb2	
<i>C. oligospora</i>	HMAS 290895	OP205473	N/A	OP205322	N/A	N/A	Zeng and Zhuang (2022)
<i>C. pallens</i>	PAD S00004	MT554899	N/A	N/A	N/A	N/A	Forin et al. (2020)
<i>C. palmae</i>	CBS 119.87	OQ910650	OQ911009	OQ982681	OQ944662	OQ927719	Zhao et al. (2023)
<i>C. parasporodochialis</i>	CBS 192.93	OQ910651	OQ911010	OQ982682	OQ944663	OQ927720	Zhao et al. (2023)
<i>C. penicillata</i>	CBS 729.87	OQ910654	OQ911013	OQ982685	OQ944666	OQ927722	Zhao et al. (2023)
<i>C. pilosella</i>	CLLG19028	N/A	NG_153902	N/A	N/A	N/A	Lechat et al. (2020)
<i>C. pityrodes</i>	CBS 102033	AF210672	OQ911014	AF358212	N/A	OQ927723	Schroers (2001)
<i>C. pnagiana</i>	CLLG19041	N/A	NG_153903	N/A	N/A	N/A	Lechat et al. (2020)
<i>C. pseudochroleuca</i>	CBS 192.94	AF358238	N/A	AF358171	N/A	N/A	Schroers (2001)
<i>C. pseudostriata</i>	CBS 309.96	OQ910673	OQ911032	OQ982704	OQ944685	OQ927741	Zhao et al. (2023)
<i>C. pseudostriatopsis</i>	h116	N/A	N/A	AB237465	N/A	N/A	Hirooka and Kobayashi (2007)
<i>C. ralfsii</i>	CBS 129.87	AF210676	N/A	AF358195	N/A	N/A	Schroers (2001)
<i>C. reniformis</i>	CBS 695.86	OQ910685	OQ911044	OQ982714	OQ944697	OQ927753	Zhao et al. (2023)
<i>C. rhinolophicola</i>	KUMC 21-0438	ON426841	N/A	OR025936	N/A	N/A	Liu et al. (2023)
<i>C. rhinolophicola</i>	HKAS122257	ON426840	N/A	OR025937	N/A	N/A	Liu et al. (2023)
<i>C. rhizophaga</i>	CBS 202.37	AF358225	MH867396	AF358156	N/A	N/A	Schroers (2001)
<i>C. rogersoniana</i>	CBS 582.89	AF210691	N/A	AF358189	N/A	N/A	Schroers (2001)
<i>C. rosea</i>	CBS 1221.71	DQ674381	OQ911077	OQ982747	OQ944730	OQ927786	Zhao et al. (2023)
<i>C. samuelsii</i>	CBS 699.97	OQ910812	N/A	AF358190	N/A	N/A	Zhao et al. (2023)
<i>C. setosa</i>	CBS 834.91	AF210670	N/A	AF358211	N/A	N/A	Schroers (2001)
<i>C. solani</i>	CBS 101924	AF358232	OQ911196	AF358180	OQ944847	OQ927902	Schroers (2001)
<i>C. spinulosa</i>	MFLU 17-0131	ON230049	N/A	ON238009	N/A	N/A	Perera et al. (2023)
<i>C. sporodochialis</i>	CBS 101921	AF210685	N/A	AF358149	N/A	N/A	Schroers (2001)
<i>C. squamuligera</i>	PAD S00020	MT554908	N/A	N/A	N/A	N/A	Forin et al. (2020)
<i>C. squamuligera</i>	PAD S00021	MT554909	N/A	N/A	N/A	N/A	Forin et al. (2020)
<i>C. subquaternata</i>	CBS 100003	MT537603	N/A	N/A	N/A	N/A	Forin et al. (2020)
<i>C. vacuolata</i>	CBS 191.93	OQ910868	OQ911227	N/A	OQ944876	OQ927931	Zhao et al. (2023)
<i>C. venezuelae</i>	CBS 107.87	OQ910869	OQ911228	OQ982884	OQ944877	OQ927932	Zhao et al. (2023)
<i>C. vesiculosa</i>	HMAS 183151	NR_119828	HM050302	N/A	N/A	N/A	Luo and Zhuang (2010)
<i>C. viticola</i>	CAA 944	MK156282	N/A	MK156290	MK156286	N/A	Torcatto et al. (2020)
<i>C. viticola</i>	MFLU 18-2770	MT215573	MT396164	N/A	MT212204	N/A	Perera et al. (2020)
<i>C. wenpingii</i>	HMAS 172156	NR_119651	MH874867	N/A	N/A	N/A	Luo and Zhuang (2007)
<i>C. yadongensis</i>	HKAS 133183	PQ522508	PQ634395	PQ650463	PQ650481	PQ538524	present study
<i>C. yadongensis</i>	HKAS 133184	PQ522509	PQ634396	PQ650464	PQ650482	PQ538525	present study
<i>C. zelandiaenovae</i>	CBS 100979	AF358229	OQ911231	N/A	OQ944880	OQ927935	Schroers (2001)
<i>C. zelandiaenovae</i>	CBS 232.80	AF210684	N/A	AF358185	N/A	N/A	Schroers (2001)
<i>Mycocitrus coccicola</i>	HD 2016	KU720552	KU720545	N/A	N/A	N/A	Dao et al. (2016)
<i>M. coxeniae</i>	BRIP 49559a	OQ629341	N/A	N/A	N/A	N/A	Zhao et al. (2023)
<i>Sesquicillium aquaticum</i>	HKAS 125804	OP876724	OP875077	N/A	N/A	N/A	Bao et al. (2023)
<i>S. buxi</i>	CBS 696.93	AF210667	KM231721	AF358215	KM231977	KM232416	Schroers (2001)
<i>S. candelabrum</i>	CBS 504.67	AF210668	N/A	N/A	N/A	N/A	Schroers (2001)
<i>S. candelabrum</i>	YFCC 896	MW199067	N/A	MW201674	N/A	N/A	Wang et al. (2023)
<i>S. essexcoheniae</i>	BRIP 75170a	OQ629342	N/A	N/A	OQ944511	OQ914830	Zhao et al. (2023)
<i>S. phyllophila</i>	CBS 921.97	NR_137531	N/A	N/A	N/A	N/A	Schroers (2001)
<i>S. rossmaniae</i>	CBS 210.93	AF358227	N/A	AF358213	N/A	N/A	Vu et al. (2019)
<i>S. saulense</i>	BRFM 2782	MK635054	N/A	N/A	N/A	N/A	Lechat et al. (2020)
<i>S. sesquicillii</i>	CBS 180.88	AF210666	NG_228796	AF358214	OQ944535	N/A	Schroers (2001)
<i>S. shanghaiense</i>	HMAS 351878	OL897002	OL897044	N/A	N/A	N/A	Zhang et al. (2023)
<i>S. shanghaiense</i>	GZUIFR 21.916	OL897003	OL897045	N/A	N/A	N/A	Zhang et al. (2023)
<i>Fusarium acutatum</i>	CBS 402.97	NR_111142	N/A	MT011051	N/A	N/A	Luo and Zhuang (2007)
<i>Nectria cinnabarina</i>	CBS 279.48	AF163025	HM484754	HM484802	HM484649	N/A	Hirooka et al. (2011)

The newly generated sequences are in red. The type strains are indicated in bold. The synonymizing are indicated in green. N/A denotes the unavailable data in GenBank.

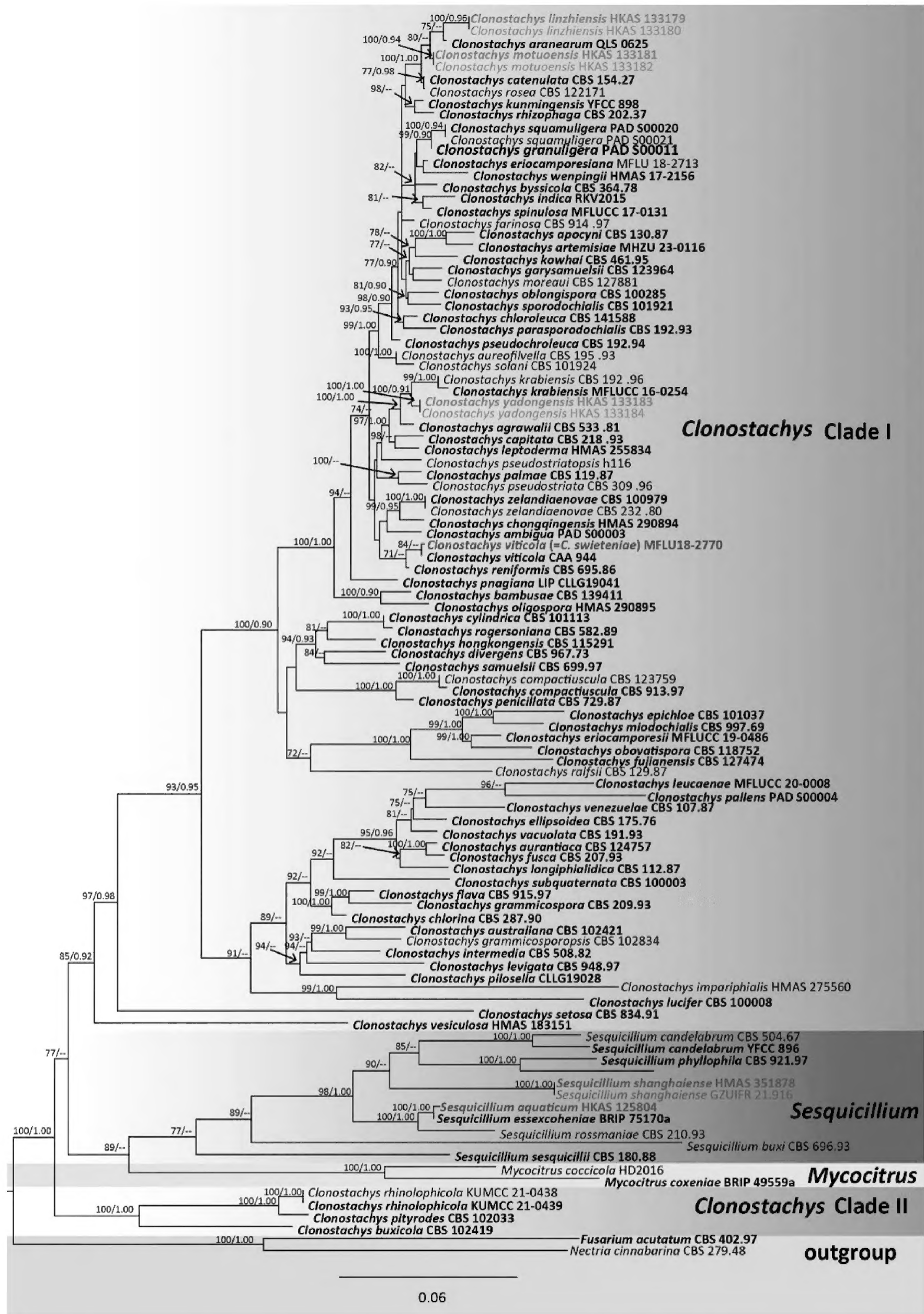


Figure 1. Phylogenetic tree generated from maximum likelihood analysis based on a combined 28S *tef1*, *rpb2*, ITS and *tub2* sequence dataset. Bootstrap support values for ML equal to or greater than 70% and PP equal to greater than 0.90 are indicated at the nodes as ML/PP. The ex-type strains are in bold, while the new isolates are in red, and the synonymizing taxa are indicated in green.

Taxonomy

Clonostachys

***Clonostachys* Corda, Pracht-Fl. Eur. Schimmelbild: 31 (1839)**

Index Fungorum: IF7701

Facesoffungi Number: FoF02102

Classification. Bionectriaceae, Hypocreales, Sordariomycetes.

Morphological characteristics. **Sexual morph:** **Ascomata** perithecial. **Perithecia** superficial, solitary to gregarious, subglobose to globose, papillate or non-papillate, no colour change in 3% KOH or 100% LA. **Asci** clavate to subcylindrical, 6–8-spored. **Ascospores** ellipsoidal to oblong ellipsoidal, uniseptate, hyaline, smooth-walled, uniseriate or irregular biseriate. **Asexual morph:** Hyphomycetous. **Conidiophores** dimorphic or monomorphic, sporodochial, synnematosus, hyaline, brown or blackish brown. **Phialides** phialidic, cylindrical to flask-shaped. **Conidia** aseptate, hyaline, smooth, ovoid to ellipsoid.

Type species. *Clonostachys araucaria* Corda, Pracht-Fl. Eur. Schimmelbild.: 31 (1839)

Notes. *Clonostachys* is the second largest genus in Bionectriaceae, with 130 epithets (Index Fungorum 2025). Several members of *Clonostachys* are ecologically and economically important (Abeywickrama et al. 2023). Some *Clonostachys* spp. are destructive, including parasitic in myxomycetes, nematodes, ticks, molluscs, and leafhoppers (Schroers 2001; Toledo et al. 2006; Perera et al. 2023). *Clonostachys rosea* and *C. catenulata* are reported as destructive to ascomycetes and basidiomycetes (Schroers 2001; Chatterton et al. 2008) and *C. chuyangsinensis* and *C. araneorum* have been reported as spider-pathogenic fungi (Wan et al. 2016; Wang et al. 2023).

Clonostachys rosea has been studied as a potential biological control agent for various plant diseases and pests such as strawberry gray mold (Cota et al. 2008), *Fusarium* head blight of wheat (Xue et al. 2008), and *Pythium tracheiphilum* in Chinese cabbage (Møller et al. 2003). Several closely related species to *Clonostachys rosea*, such as *C. byssicola*, *C. chloroleuca*, *C. rhizophaga*, and *C. solani* also possess biocontrol properties (Mendoza García et al. 2003; Krauss et al. 2013; Sun et al. 2017; Broberg et al. 2021).

***Clonostachys linzhiensis* S.C. He, K.D. Hyde & Q. Zhao, sp. nov.**

Index Fungorum: IF902917

Facesoffungi Number: FoF16789

Fig. 2

Etymology. The species epithet is derived from Linzhi City, where the holotype was collected.

Typification. CHINA • Xizang Autonomous Region, Linzhi City, Motuo County (29°11'N, 95°8'E, 1561 m), on the lower part of the leaves of *Houttuynia cordata*, July 27, 2022, collected by Hong-De Yang, YHD691 (**holotype**: KUN-HKAS 133179); ex-type living culture: KUNCC24-18528). GenBank: ITS: PQ522504, 28S: PQ634391, *tef1*: PQ650477, *tub2*: PQ650459.

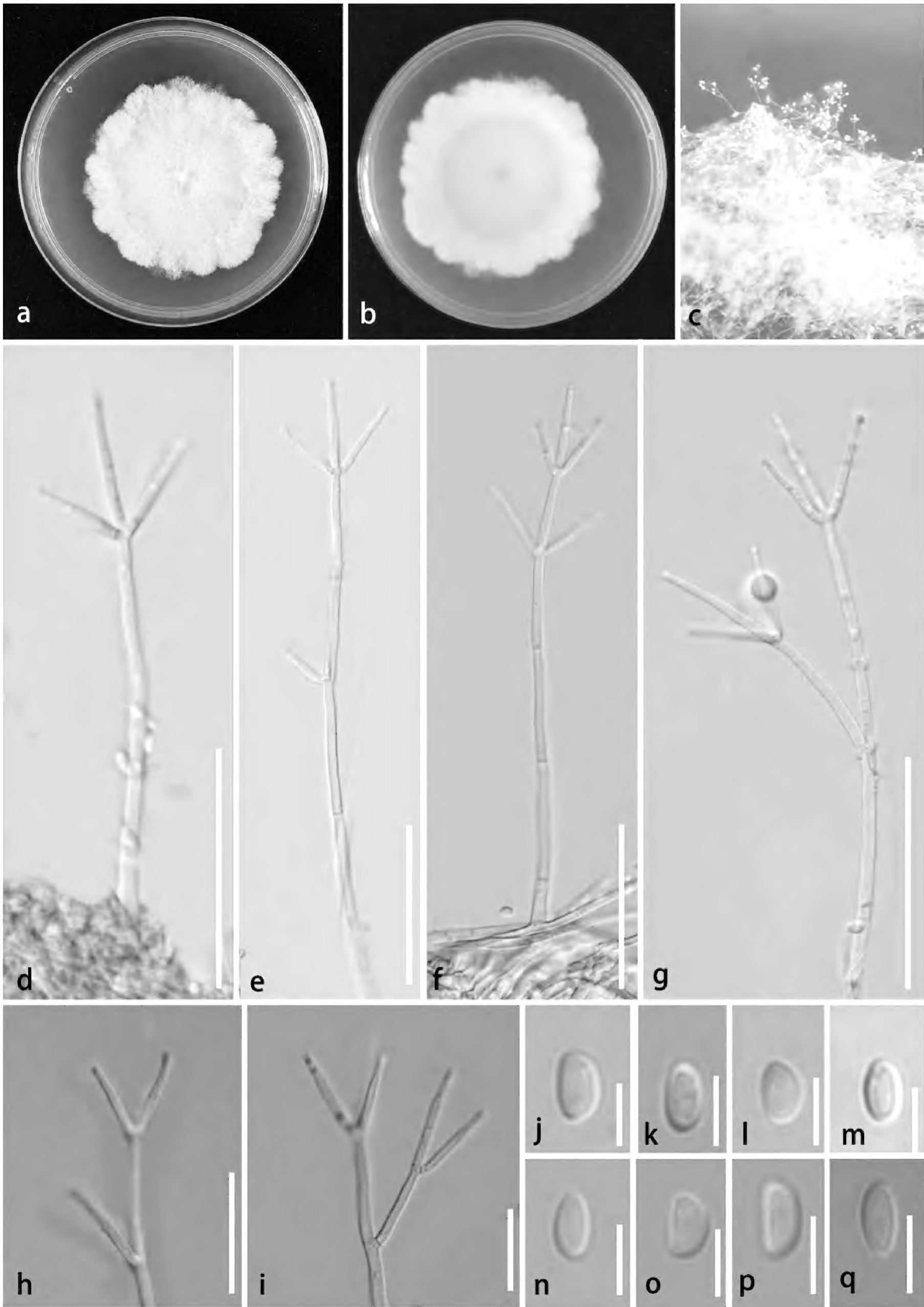


Figure 2. *Clonostachys linzhiensis* (HKAS 133179, Holotype) **a, b** culture on PDA (**a** above **b** below) **c** colonies on WA **d–g** conidiophores **h, i** phialides **j–q** conidia. Scale bars: 50 µm (**d–g**); 50 µm (**h, i**); 5 µm (**j–q**).

Description. **Sexual morph:** Not observed. **Asexual morph:** Hyphomycetous. **Colonies** on the WA, raised, medium sparse, rough, white at apex. **Conidiophores** mononematous, erect, simple, verticillium-like, straight or flexuous, branched, smooth-walled, thin-walled, septate, hyaline, produce globose cells at the apex, terminal branches developing into phialides, $110\text{--}232 \times 2.5\text{--}3.9 \mu\text{m}$ ($\bar{x} = 170 \times 3.2 \mu\text{m}$, $n = 20$). **Phialides** polytretic, terminal on branches, phialides cylindrical but slightly tapering towards the tips, aseptate, hyaline, smooth, thin-walled, terminal developing into conidia, $15.3\text{--}23.8 \times 1.5\text{--}3.3 \mu\text{m}$ ($\bar{x} = 19.8 \times 2.2 \mu\text{m}$, $n = 20$). **Conidia** amerospores, solitary, acrogenous, simple, doliform to ellipsoidal, smooth, thin-walled, aseptate, hyaline, $3.9\text{--}5.7 \times 2.2\text{--}3.2 \mu\text{m}$ ($\bar{x} = 4.7 \times 2.6 \mu\text{m}$, $n = 30$).

Culture characteristics. Colonies on PDA reaching 5.0–5.5 cm after 20 days of incubation at 25 °C, white above, pale yellow reverse, medium sparse, concave in the center, convex around, hairy, lobate, velvety, ciliate, not pigment produced.

Habitat. Leaves of *Houttuynia cordata*.

Additional material examined. CHINA • Xizang Autonomous Region, Linzhi City, Motuo County (29°11'N, 95°8'E, 1561 m), on the lower part of the leaves of *Houttuynia cordata*, July 27, 2022, collected by Hong-De Yang, HSC983 (**isotype:** KUN-HKAS 133180); ex-isotype living culture: KUNCC24-18529). GenBank: ITS: PQ522505, 28S: PQ634392, *tef1*: PQ650478, *tub2*: PQ650460.

Notes. In the phylogenetic analysis, *Clonostachys linzhiensis* shared a close phylogenetic relationship with *C. araneorum* and *C. motuoensis* (Fig. 1). *Clonostachys linzhiensis* shares similar morphology to *C. araneorum* and *C. motuoensis* in having mononematous, erect, verticillium-like conidiophores that are straight or flexuous, smooth-walled, hyaline, phialides are polytretic, terminal, flask-shaped, aseptate, hyaline, smooth and the conidia are amerospores, acrogenous, ellipsoidal, aseptate, hyaline (Wan et al. 2016). However, *Clonostachys linzhiensis* (HKAS 133179 and HKAS 133180) has larger conidiophores (L/W ratio: 53 vs 12 and L/W ratio: 53 vs 35) and longer phialides (L/W ratio: 9 vs 6.7 and L/W ratio: 9 vs 4.7) in comparison to *C. araneorum* and *C. motuoensis*. Furthermore, the ITS and *tub2* sequence of *Clonostachys linzhiensis* differs from *C. araneorum* which revealed 13/510 (2.5%) and 7/291 (2.4%) base pair differences, respectively. Based on the differences in morphology (larger conidiophores and longer phialides) and phylogeny, along with the guidelines of Maharachchimbukura et al. (2021), we identify our specimen as a new species, *C. linzhiensis*.

***Clonostachys motuoensis* S.C. He, K.D. Hyde & Q. Zhao, sp. nov.**

Index Fungorum: IF902918

Facesoffungi Number: FoF16790

Fig. 3

Etymology. The species epithet is derived from the location “Motuo County”, from where the holotype was collected.

Typification. CHINA • Xizang Autonomous Region, Linzhi City, Motuo County (29°11'N, 95°8'E, 1561 m), on the lower part of the leaves of *Houttuynia cordata*, July 27, 2022, collected by Hong-De Yang, YHD669-1 (holotype: KUN-HKAS HKAS 133181); ex-type living culture: KUNCC24-18530). GenBank:ITS: PQ522506, 28S: PQ634393, *tef1*: PQ650479, *tub2*: PQ650461.

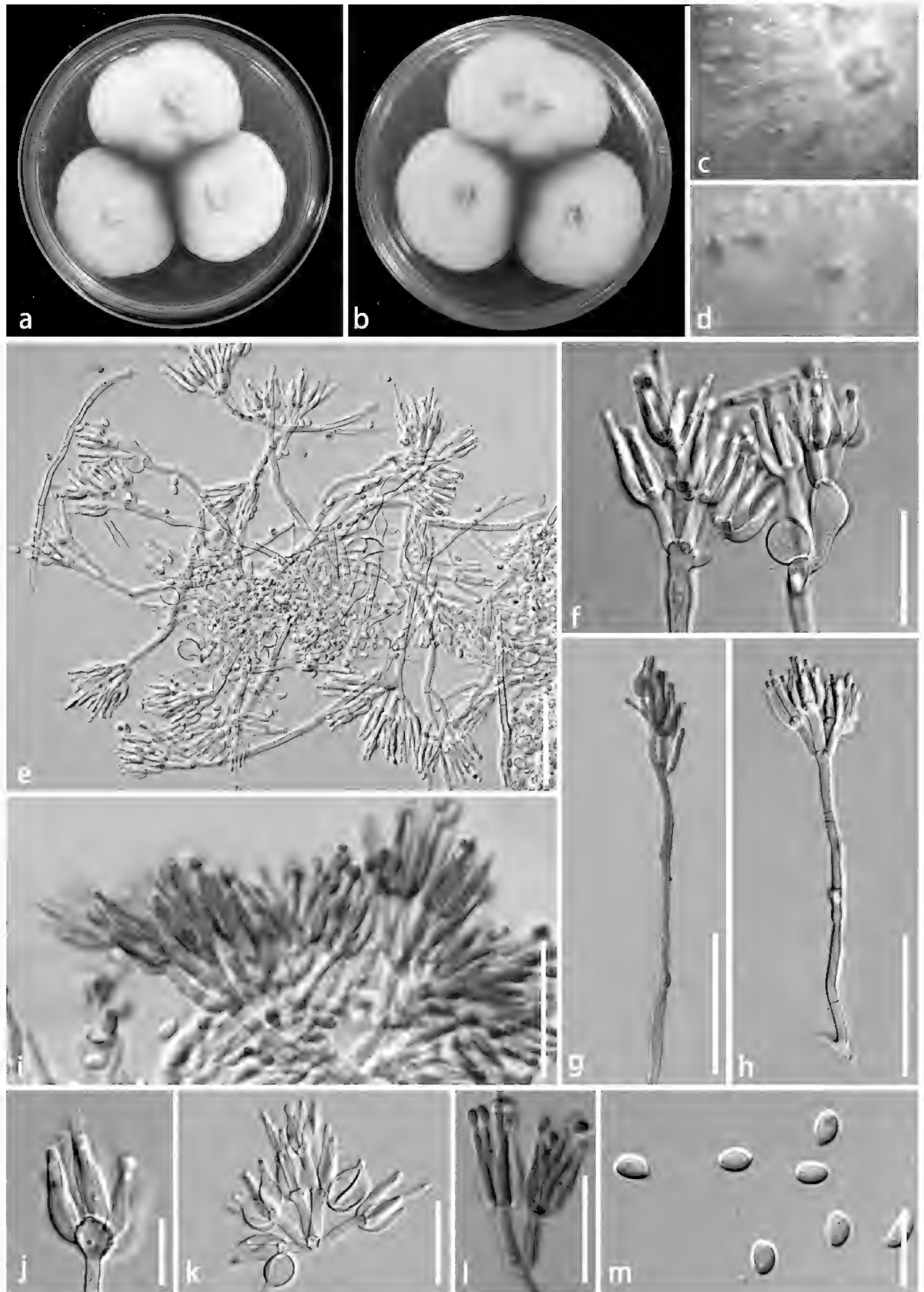


Figure 3. *Clonostachys motuoensis* (HKAS 133181, Holotype) **a, b** culture on PDA (**a** above **b** below) **c, d** colonies on WA **e–h** conidiophores and conidiophores apex **i–l** phialides **m** conidia. Scale bars: 50 μm (**e, g, h**); 25 μm (**f, i, k, l**); 10 μm (**j, m**).

Description. **Sexual morph:** Not observed. **Asexual morph:** Hyphomycetous. **Colonies** on the WA, solitary or gregarious, white to pale yellow, raised, dense, rough. **Conidiophores** mononematous, penicillate, straight or flexuous, branched at the apex, smooth, thin-walled, septate, hyaline, conidiophores produce globose cells at the apex, from globose to elongated or continue to differentiate, terminal branches developing into phialides, $94\text{--}146 \times 2.5\text{--}4.7 \mu\text{m}$ ($\bar{x} = 125 \times 3.5 \mu\text{m}$, $n = 20$). **Phialides** monophialidic, terminal, flask-shaped, aseptate, hyaline, smooth, thin-walled, terminal developing into conidia, $9.1\text{--}18.7 \times 2.3\text{--}3.5 \mu\text{m}$ ($\bar{x} = 13.2 \times 2.8 \mu\text{m}$, $n = 20$). **Conidia** amerospores, solitary, acrogenous, simple, ellipsoidal to oblong with obtuse ends, smooth, thin-walled, aseptate, hyaline, minutely guttulate, $3.9\text{--}5.6 \times 2.5\text{--}3.3 \mu\text{m}$ ($\bar{x} = 4.6 \times 2.9 \mu\text{m}$, $n = 30$).

Culture characteristics. Colonies on PDA reaching 3.5–4 cm after 20 days of incubation at 25 °C, white both above and reverse, medium sparse, raised, smooth, fimbriate, velvety, ciliate, not pigment produced.

Habitat. Leaves of *Houttuynia cordata*.

Additional material examined. CHINA • Xizang Autonomous Region, Linzhi City, Motuo County (29°11'N, 95°8'E, 1561 m), on the lower part of the leaves of *Houttuynia cordata*, July 27, 2022, collected by Hong-De Yang, HSC986 (isotype: KUN-HKAS 133182); ex-isotype living culture: KUNCC24-18531). GenBank: ITS: PQ522507, 28S: PQ634394, *tef1*: PQ650480, *tub2*: PQ650462.

Notes. In the phylogenetic analysis, *Clonostachys motuoensis* clustered sister to *C. linzhiensis* and *C. araneorum* (Fig. 1). Morphologically, our specimen (HKAS 133181 and HKAS 133182) has larger conidiophores (L/W ratio: 35 vs 12) and longer phialides (L/W ratio: 4.7 vs 6.7) in comparison to *C. araneorum*. *Clonostachys motuoensis* differs from *C. araneorum* by 6/544 (1%) ITS and 4/294 (1.3%) *tub2* differences in the nucleotides. It is worth noting that *C. araneorum* is parasitic on spiders, while *C. motuoensis* is endophytic on *Houttuynia cordata* leaves. In addition, *C. araneorum* was collected from Qian Ling Shan Park, Guiyang City, Guizhou Province, China, with an altitude of 1100–1369 m, belonging to a plateau subtropical climate (Wan et al. 2016). *Clonostachys motuoensis* was collected from Motuo County, Linzhi City, Xizang Autonomous Region, China, with an altitude of 1561 m, belonging to a tropical rainforest climate. Based on these distinctions and following the guidelines of Maharachchimbukura et al. (2021), we identified our specimen as a new species, *C. motuoensis*.

***Clonostachys yadongensis* S.C. He, K.D. Hyde & Q. Zhao, sp. nov.**

Index Fungorum: IF902919

Facesoffungi Number: FoF16791

Fig. 4

Etymology. The species epithet is derived from Yadong County, where the holotype was collected.

Typification. CHINA • Xizang Autonomous Region, Linzhi City, Yadong County (27°48'N, 88°83'E, 3894 m), on the lower part of the leaves of *Ageratina adenophora* leaves, July 24, 2023, collected by Shu-Cheng He, HSC1025 (holotype: KUN-HKAS 133183); ex-type living culture: KUNCC24-18532). GenBank: ITS: PQ522508, 28S: PQ634395, *tef1*: PQ650481, *tub2*: PQ650463, *rpb2*: PQ538524.

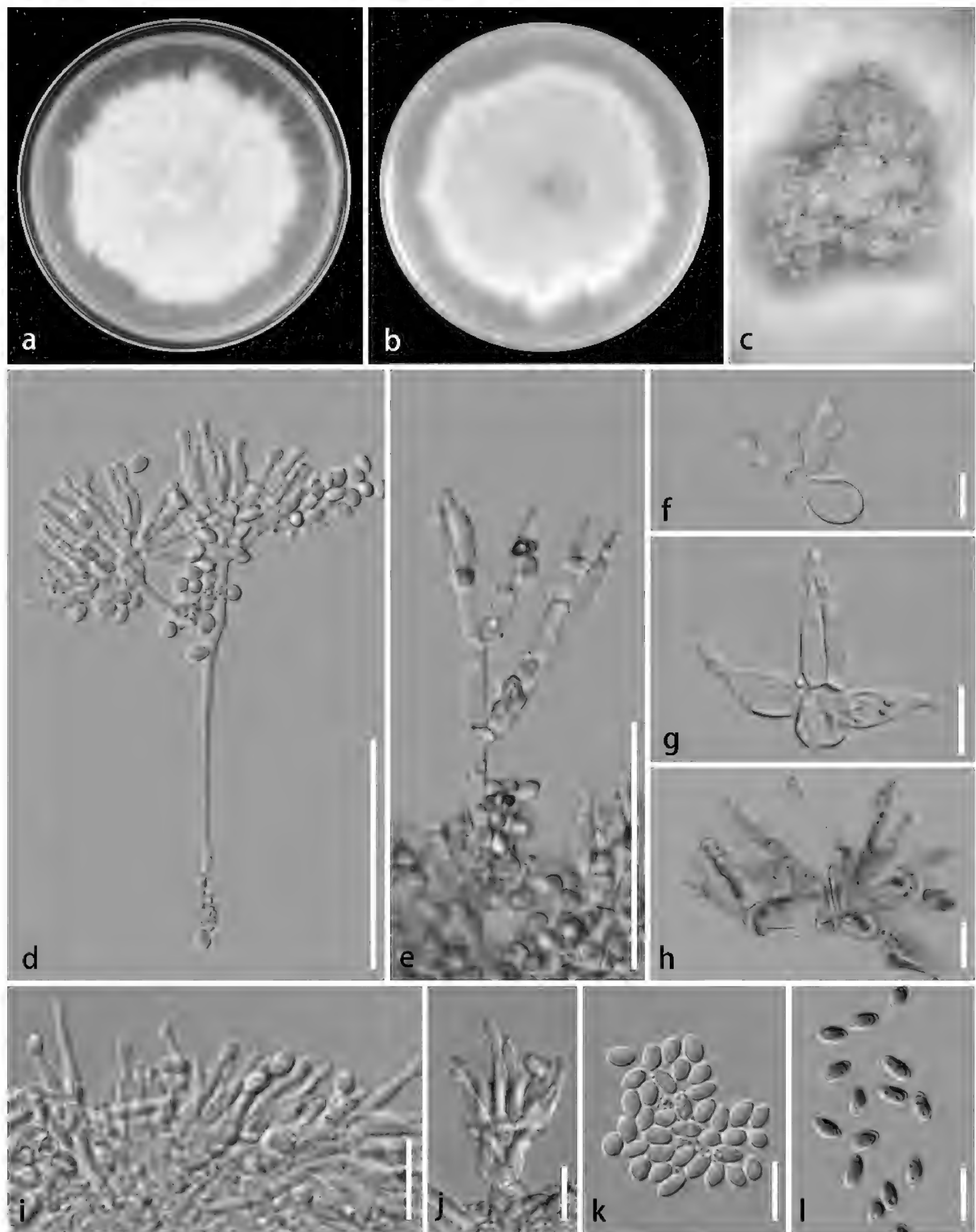


Figure 4. *Clonostachys yadongensis* (HKAS 133183, Holotype) **a, b** culture on PDA (**a** above **b** below); **c** colonies on WA **d–h** conidiophores **f–j** phialides **k, l** conidia. Scale bars: 50 µm (**d–f**); 20 µm (**g–l**).

Description. Sexual morph: Not observed. **Asexual morph:** Hyphomycetous. **Colonies** on the WA, solitary or gregarious, white to pale yellow, raised, medium sparse, rough. **Conidiophores** mononematous, penicillate, straight or flexuous, branched, smooth-walled, thin-walled, septate, hyaline, produce globose cells at the apex, terminal branches developing into phialides, $80\text{--}118 \times 2.4\text{--}3.4 \mu\text{m}$ ($\bar{x} = 97 \times 2.8 \mu\text{m}$, $n = 20$). **Phialides** polyblastic, terminal, flask-shaped, aseptate,

hyaline, smooth, thin-walled, minutely guttulate, terminal developing into conidia, $9.6\text{--}15.6 \times 1.7\text{--}2.3 \mu\text{m}$ ($\bar{x} = 13.1 \times 2 \mu\text{m}$, $n = 20$). **Conidia** amerospores, solitary, acrogenous, simple, oval to ellipsoidal, smooth, thin-walled, aseptate, hyaline, minutely guttulate, $3.6\text{--}5.4 \times 2.6\text{--}3.3 \mu\text{m}$ ($\bar{x} = 4.5 \times 2.9 \mu\text{m}$, $n = 30$).

Culture characteristics. Colonies on PDA reaching 5.5–6 cm after 20 days of incubation at 25 °C, white above, pale yellow reverse, medium sparse, raised, hairy, fimbriate, velvety, ciliate, not pigment produced.

Habitat. Leaves of *Ageratina adenophora*.

Additional material examined. CHINA • Xizang Autonomous Region, Linzhi City, Yadong County (27°48'N, 88°83'E, 3894 m), on the lower part of the leaves of *Ageratina adenophora*, July 24, 2023, collected by Shu-Cheng He, HSC1025A (isotype: KUN-HKAS 133184; ex-isotype living culture: KUNCC24-18533). GenBank:ITS: PQ522509, 28S: PQ634391, *tef1*: PQ650482, *tub2*: PQ650464, *rpb2*: PQ538525.

Notes. In the phylogenetic analysis, *Clonostachys yadongensis* clustered with *C. krabiensis* with 100% MLB and 0.91 BYPP support (Fig. 1). *Clonostachys krabiensis* was introduced by Tibpromma et al. (2018) and is characterized by solitary, superficial, globose to subglobose, orange to brownish orange ascomata, 6–8-spored, cylindrical to clavate asci; fusoid to ellipsoidal, hyaline, with longitudinal striations, granulate ascospores. Its morphology fits well with the generic concept of *Clonostachys* sexual morph (Bao et al. 2023; Perera et al. 2023; Zhao et al. 2023). Our specimen (HKAS 133183) exhibited an asexual morph that is characterized by mononematous, penicillate, erect conidiophores; flask-shaped or cylindrical, aseptate, hyaline phialides; acrogenous, ellipsoidal or oblong with obtuse ends, hyaline conidia. The 28S and ITS sequences of *Clonostachys yadongensis* differ from that of *C. krabiensis* which showed base pair differences, 3/825 (0.35%), 11/513 and (2.1%) respectively. *Clonostachys krabiensis* was reported in Papua New Guinea and Thailand as a saprobe on *Pandanus* sp. and wood litter, while *C. yadongensis* was reported in the Xizang Autonomous Region, China, mainly as an endophyte on *Ageratina adenophora*. *Clonostachys krabiensis* has been reported to have a sexual morph, but *C. yadongensis* has only been observed in its asexual morph. Based on base pair differences and following the guidelines of Maharachchimbukura et al. (2021), we identified our specimen as a new species, *Clonostachys yadongensis*.

***Clonostachys viticola* C. Torcato & A. Alves, *Int. J. Syst. Evol. Microbiol.* 6 (2020)**

Index Fungorum: IF835021

Facesoffungi Number: FoF16792

Basionym. *Clonostachys swieteniae* R.H. Perera, E.B.G. Jones & K.D. Hyde, *Mycosphere* 11(1): 2135 (2020)

Description and illustration. Perera et al. 2020 and Torcato et al. 2020.

Notes. In the multigene phylogenetic analyses, *Clonostachys viticola* with *C. swieteniae*, forms a monophyletic clade in *Clonostachys*. The taxa in this clade show low genetic differences. Thus, we recommend treating *C. viticola* and *C. swieteniae* as conspecific. *Clonostachys viticola* was established by Torcato et al. (2020) from the root of *Vitis vinifera* in a terrestrial habitat of Peru (Torcato et al. 2020) and *Clonostachys swieteniae* was established by Perera et al. (2020)

from decaying fruits of *Swietenia mahagoni* in a terrestrial habitat of Thailand (Perera et al. 2020). Morphologically, *C. viticola* with *C. swieteniae* are highly similar, but there are minor differences in phialides ($13.1 \times 2.1 \mu\text{m}$ vs $11.4 \times 2.6 \mu\text{m}$), and conidia ($5.6 \times 2.9 \mu\text{m}$ vs $6 \times 2.2 \mu\text{m}$). Through base pair comparison, the ITS and *tef1* sequence of *Clonostachys viticola* differs from that of *C. swieteniae* in 0/500 (0%) and 3/406 (0.7%), respectively. The results indicate that different environments have shaped the morphology (Bhunjun et al. 2022; Hyde et al. 2020b; Phukhamsakda et al. 2022). *Clonostachys viticola* was published prior to *C. swieteniae*. Therefore, we propose *C. swieteniae* as a synonym of *C. viticola*.

New combinations of *Sesquicillium*

***Sesquicillium* W. Gams, Acta bot. neerl. 17(6): 455 (1968)**

Index Fungorum: IF9906

Facesoffungi Number: FoF16793

Classification. Bionectriaceae, Hypocreales, Sordariomycetes

Morphological characteristics. **Sexual morph:** Ascomycetous. **Perithecia** solitary, gregarious or loosely aggregated, globose to subglobose, 200–400 μm diam, pale yellow or pale to light orange, not papillate, Perithecial wall either consisting of two or one major wall regions. **Asci** clavate, 8-spored, with flat or rounded apex. **Ascospores** aseptate or 1-septate, hyaline, spinulose, warted, with short striae, ellipsoidal to fusiform. **Asexual morph.** Hyphomycetous. **Conidiophores** macronematous, mononematous, monomorphic or dimorphic, penicillate, verticillate; branches at apex. **Phialides** one or two successive intercalary phialides, terminal, terminal whorls consisting of narrowly flask-shaped, hyaline. **Conidia** obovoid, ellipsoid, or fusoid, slightly curved or straight, hyaline, aseptate, smooth-walled, thin-walled.

Type species. *Sesquicillium buxi* (J.C. Schmidt ex Link) W. Gams, Acta bot. neerl. 17(6): 455 (1968)

Notes. *Sesquicillium* was established by Gams (1968). Morphologically, *Sesquicillium* shares similar characteristics with *Clonostachys* in that the conidiophores are macronematous, monomorphic or dimorphic, penicillate, verticillate-like, branched, flask-shaped conidiogenous cells (Preedanon et al. 2023; Zhao et al. 2023). Zhao et al. (2023) revealed the close relationship between *Clonostachys* and *Sesquicillium* and reclassified eight species of *Clonostachys* to *Sesquicillium*. The difference between *Sesquicillium* and *Clonostachys* lies in the development of their conidiophores. In *Sesquicillium*, the conidiophore will form a lateral conidia process after bifurcation, leading to the production of conidia. In *Clonostachys*, the conidiophore will not form lateral conidia protrusions after bifurcation. It continues to differentiate into terminal phialides (Gams 1968; Schroers 2001). Based on the research of Chen et al. (2023), and Zhao et al. (2023), we used ITS, 28S, *tef1*, *tub2*, and *rpb2* to reconstruct a phylogenetic tree to investigate the relationship of *Clonostachys* species. The results show that *Clonostachys aquatica* and *C. shanghaiensis* are far from *Clonostachys* and more closely related to *Sesquicillium*. Therefore, based on morphological and phylogenetic analysis, we propose *C. aquatica* and *C. shanghaiensis* are synonyms of *S. aquaticum* and *S. shanghaiense*.

***Sesquicillium aquaticum* (D.F. Bao, K.D. Hyde & Z.L. Luo) S.C. He, K.D. Hyde & Jayaward, [as 'aquatica'], comb. nov.**

Index Fungorum: IF903022

Facesoffungi Number: FoF16794

Basionym. *Clonostachys aquatica* D.F. Bao, K.D. Hyde & Z.L. Luo, *Fungal Diversity*, (2023).

Holotype. HKAS 125804.

Description and illustration. See Bao et al. 2023.

Notes. *Clonostachys aquatica* was established by Bao et al. (2023) based on ITS and *tub2* sequence data (holotype HKAS 125804). Through the study of Bao et al. (2023), *C. aquatica* clustered as a clade sister to *C. rossmaniae* with strong support (94% MLB, 98% MYPP). Following Bao et al. (2023), we added 28S, *tef1* and *rpb2* sequence data, and the results showed that *C. aquatica* clustered with *Sesquicillium essexcoheniae* (100% MLB, 0.97 BYPP), forming a successive sister clade with *S. rossmaniae* (99% MLB, 1.00 BYPP) (Fig. 1). *Clonostachys aquatica* shows a closer relationship with *Sesquicillium* in phylogenetic analysis. Therefore, based on phylogenetic analysis, we propose *C. aquatica* as a synonym of *S. aquaticum*.

***Sesquicillium shanghaiense* (Zhi Yuan Zhang, Y.F. Han & Z.Q. Liang) S. C. He, K.D. Hyde & Jayaward, [as 'shanghaiensis'], comb. nov.**

Index Fungorum: IF903023

Facesoffungi Number: FoF16795

Basionym. *Clonostachys shanghaiensis* Zhi Yuan Zhang, Y.F. Han & Z.Q. Liang, *MycKeys* 98: 198 (2023).

Holotype. HMAS 351878.

Description and illustration. Zhang et al. (2023).

Notes. *Clonostachys shanghaiensis* was established by Zhang et al. (2023), based on ITS and *tub2* sequence data (HMAS 351878). *Clonostachys shanghaiensis* clustered as a sister clade to *C. rossmaniae* (95% MLB, 0.99 BYPP) (Zhang et al. 2023). In this study, phylogenetic analysis showed that *Clonostachys shanghaiensis* formed a successive sister clade with *S. phyllophila*, *S. saulensis*, and *S. candelabrum* (Fig. 1). It is worth noting that *S. phyllophila*, *S. saulense*, and *S. candelabrum* were renamed by Zhao et al. (2023) as *C. phyllophila* (Schroers 2001), *C. saulensis* (Lechat et al. 2020), *C. candelabrum* (Schroers 2001) and *C. chuyangsinensis* (Wang et al. 2023) based on morphology and phylogenetic analysis. Therefore, based on phylogenetic analysis, we propose *C. shanghaiensis* as a synonym of *S. shanghaiense*.

Discussion

Rossmann et al. (2001) studied the asexual species in 15 genera of Bionectriaceae (Hypocreales) using 28S sequence data and showed that Bionectriaceae formed a monophyletic group. Recently, additional DNA gene sequences such as *act1*, *tub2*, *rpb1*, and *tef1* have been used to enhance the precision of phylogenetic trees within the *Clonostachys/Bionectria* species (Moreira et al. 2016). However, available sequence data for these four protein-encoding gene regions

is lacking in GenBank (Moreira et al. 2016). Wang et al. (2023), stated that *tef1* sequence data showed the highest resolution for distinguishing *Clonostachys* species (*tef1*>*tub2*>ITS) based on the investigation conducted for genetic divergence comparisons of *Clonostachys*. Zhao et al. (2023) investigated the generic delineation with broad taxon sampling with morphology and multi-gene (ITS, 28S, *tef1*, *tub2*, *rpb2*) phylogenetic analysis and found a close relationship to *Sesquicillium*. Further, *Sesquicillium* was resurrected to accommodate the former subgenera *Epiphloea* and *Uniparietina* (Zhao et al. 2023). We constructed a phylogenetic tree (Fig. 1) of *Clonostachys* based on five genes (28S, *tef1*, *rpb2*, ITS, and *tub2*) and show that *Clonostachys*/*Bionectria* form a similar topology with Perera et al. (2023). However, as with other studies, we did not achieve a well-supported clade, as some but not all subgenera are mono- or paraphyletic (Moreira et al. 2016; Bao et al. 2023; Perera et al. 2023; Wang et al. 2023; Zhao et al. 2023). Morphologically, the asexual morphs of *Clonostachys* exhibit similarities with those of *Sesquicillium* (Preedanon et al. 2023), *Penicillium* (Crous et al. 2023), *Verticillium* (Crous et al. 2022), *Gliocladium* (Rehner and Samuels 1994) acremonium-like (Preedanon et al. 2023). They typically feature macronematous, monomorphic penicillate, or dimorphic penicillate conidiophore. Based on recent studies by Bao et al. (2023), Wang et al. (2023), and Zhao et al. (2023), we have clarified the relationships within the *Clonostachys* and proposed that *C. aquatica*, *C. shanghaiensis*, and *C. swieteniae* be considered synonyms of *S. aquaticum*, *S. shanghaiense*, and *C. viticola*, respectively. *Clonostachys aquatica* and *C. shanghaiensis* were positioned in a distantly related clade (Clade II) to *Clonostachys sensu stricto*. *Mycocitrus* and *Sesquicillium*, were positioned between Clade I and II (Fig. 1). Thus, further studies are required for the phylogenetic resolution of *Clonostachys*.

Clonostachys is reported in various plant hosts: Apocynaceae, Arecaceae, Asteraceae, Boraginaceae, Buxaceae, Ericaceae, Fagaceae, Leguminosae, Melampsoraceae, Nelumbonaceae, Pandanaceae, Rosaceae, and Rutaceae (Wang et al. 2023; Jayawardena et al. 2025). Our study reported three new species from Eupatorieae (*C. yadongensis*) and Saururaceae (*C. linzhiensis* and *C. motuoensis*). *Clonostachys* species exhibit a saprobic or endophytic lifestyle, playing crucial roles in nutrient cycling and plant health (Zeng and Zhuang 2022). *Clonostachys* species are significant for their adaptability and potential as biological control agents against plant pathogens (Wang et al. 2023; Zhao et al. 2023).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

S.-C.H and V.T. conceived and designed the study. H.-D. Y provided two new species. Y.-W.Z make two plates. S.-C.H and Y.-W.Z. generated the DNA sequence data. S.-C.H analyzed the data. S.-C.H. wrote the manuscript draft. V.T., C.S.B., P.C., L.S.D., R.S.J., Q.Z., K.D.H. revised the manuscript. FO provided financial support. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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